Physiologic Assessment of Fetal Compromise: Biomarkers of Toxic Exposure

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Understanding the physiologic and endocrinologic basis of fetal development is a major goal of perinatal biology. During the past decade a number of technological developments have allowed more precise evaluation of the fetus *in utero* and diagnosis of abnormalities. Despite these methodological achievements, however, there are no specific biological markers currently available to indicate that exposure to a given xenobiotic is associated with a cellular, subcellular, or pharmacodynamic event.

This paper evaluates the following issues: What are some of the unique physiologic and endocrinologic features of the fetal *milieu intérieur?* What problems are peculiar to fetal assessment? Of what value are techniques such as ultrasonography, amniocentesis, chorionic villus sampling, fetoscopy, and fetal blood and tissue sampling for obtaining appropriate biomarkers? What are some examples of validated biomarkers and their applicability? What promising biomarkers are on the horizon? What are some of the promising techniques such as the evaluation of fetal body movements, breathing activity, electronic heart rate monitoring, and nuclear magnetic resonance? How may molecular probes be of value as biological markers of fetal compromise? What are some of the major research gaps and needs, and how should research priorities be set?

Some of these topics are addressed. Moreover, the more general role(s) that various diagnostic methods and biological markers can have in an understanding of the regulation of fetal growth and differentiation and the role of xenobiotics in affecting the normal course of events are discussed.

Introduction

Life does not begin at birth. Prenatally, we are exposed to a variety of chemicals and drugs inadvertently or deliberately administered to a pregnant woman. In rare instances, such exposure results in a malformed child. These cases naturally attract wide attention, generate compassion, and stimulate further research.

During recent years several technological developments give promise that birth defects will become even more rare. These developments include increasing sophistication in the art and science of fetal diagnosis and methodological advances in genetics, molecular biology, and toxicology, which allow even earlier and more subtle alterations from normal to be detected. As an illustration of this progress, during the past decade or so, a number of monographs have appeared, devoted to the topic of drug effects on the developing organism (1-6), and an even larger number of articles and volumes are devoted to *in utero* diagnosis (7).

Despite a number of advances that give the appearance that the field is moving quite rapidly into the twenty-first century, many would regard these devel-

opments and a deep appreciation and understanding of the molecular basis of drug- or chemical-induced developmental abnormalities as occurring too slowly. This article will review several issues, including the following: What are some of the unique features of the fetal *milieu intérieur?* What problems are peculiar to fetal assessment? Of what value are techniques such as ultrasonography, amniocentesis, chorionic villus sampling, fetoscopy, and fetal blood and tissue sampling for obtaining appropriate biomarkers? What are some examples of validated biomarkers and or their applicability? What promising biomarkers are on the horizon? What are some of the promising techniques such as the evaluation of fetal body movements, breathing activity, electronic heart rate monitoring, and nuclear magnetic resonance? How may molecular probes be of value as biological markers of fetal compromise? What are the major research gaps and needs, and how should research priorities be set?

Unique Features of the *Milieu Intérieur*

Several considerations of the differences between fetal and adult biology are relevant to a consideration of biological markers. From a cardiopulmonary stand-

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point, the fetus maintains normal tissue oxygenation in the face of what would be considered pathologically low arterial O_2 tension in the adult by maintaining a cardiac output over twice that of an adult on a per kilogram basis. Some specifics of these differences are detailed in Table 1.

The developing fetus is also unique from an endocrinologic standpoint (Table 2). In part this relates to the interaction and interdependence of the maternal-placental-fetal complex, such that the extraembryonic membranes contain certain key enzymes for the metabolism of steroids and prostaglandins that are absent or present in only very low concentrations in the fetus, while in turn the fetal adrenal and liver contain key enzymes absent from the placenta. For instance, the inner or fetal zone of the fetal adrenal cortex has a high steroid sulfokinase activity [so that a major secretory product is dehydroepiandosterone sulfate (DHEAS)], while it is deficient in $\Delta5,3\beta$-ol dehydrogenase/$\Delta4,5,3$-

Table 1. Comparisons of fetal and maternal cardiovascular functions.

Function	Fetus	Adult
Pa_{O_2} (torr)	25	100
Pa_{CO_2} (torr)	48	40
pH	7.35	7.40
V_{O_2} (mL·min.$^{-1}$·kg^{-1})	8	4
[Hb] (g·dL^{-1})	17.5	11.5
Hematocrit (%)	55	35
O_2 content (mL·dL^{-1})	16	15.4
O_2 content (mM)	7	6.7
Blood volume (mL/kg)	130	80
Descending aorta pressure (mm Hg)	45	95
Pulmonary artery pressure (mm Hg)	45	15
Cardiac output (mL·min^{-1}kg^{-1})	220[a]	100
Systemic vascular resistance	Low	High
Vascular compliance	High	Low

[a] Calculated for both right and left ventricles.

Table 2. Hormones as potential biological markers.

Chorionic gonadotropin (hCG)
Chorionic somatomammotropin (hCS),
    also called placental lactogen (also called hPL)
Other placental hormones (SP1, PP5, β_1-PAM, PAPP-A, PAPP-B)

Prolactin (PRL)
Estrogens (E_1, E_2, E_3)
Progesterone (P_4)
α-MSH
β-Endorphin
β-Lipotropin
Adrenocorticotrophic hormone (ACTH)
Cortisol (F)
Dehydroepiandosterone (DHEAS)

Renin-angiotensin
Arginine vasopressin
Arginine vasotocin
Atrial natriuretic peptides
Catecholamines (dopamine, norepinephrine, epinephrine)
Prostaglandins

Hormonal rhythms

ketosteroid isomerase (so that conversion of pregnenolone to progesterone is limited). DHEAS is transported to the placenta, where it is hydrolyzed by a steroid sulfatase and aromatized to estradiol-17β, and to the liver where it is 16-hydroxylated. Thus, DHEAS serves as a substrate for placental estrone and estradiol biosynthesis, hormones that probably are very important in mediating many of the maternal adaptations to pregnancy (8).

The fetal adrenal gland also produces relatively large amounts of cortisol, which not only is important in the maturation of the lung, pancreas, and other organs, but also initiates a cascade of hormonal events (including decreased progesterone and increased estrogen production, and increased synthesis of prostaglandins by extraembryonic tissues) involved in the initiation of labor. The stimuli for fetal adrenal hormone synthesis are not elucidated. Although fetal adrenocorticotropin (ACTH) plays a role, other pro-opiomelanocortin (POMC)-derived peptides and several growth factors are probably also important.

In addition to the features of the maternal-placental-fetal complex there are several endocrine systems unique to fetal life. These include the paraaortic chromaffin system active in catecholamine synthesis; the fetal intermediate pituitary, which secretes α-melanocyte-stimulating hormone and β-endorphin; the posterior pituitary, which secretes arginine vasotocin in addition to vasopressin; the placental production of several polypeptides [chorionic gonadotropin (hCG) and chorionic somatomammotropin (hCS), also called placental lactogen (hPL)]; and a half-dozen or so other pregnancy-specific proteins (9), including several pituitarylike hormones and neuropeptides.

The fetus develops in an environment where respiration, alimentation, and excretory functions are provided by the placenta. About one-half of its fuel supply for tissue metabolism is for anabolism, and its growth and differentiation are regulated by growth factors. Potentially, many of these hormones and other polypeptides may serve as biological markers. Certainly, various chemicals and toxins may act on the fetal hypothalamus, adrenal, placenta, and other tissues to inhibit specific enzymes and metabolic pathways, thus altering the synthesis of, and response to, various hormones and growth factors. Nonetheless, it remains to be demonstrated to what extent this inhibition occurs.

Problems Unique to the Fetus

Clearly, optimal fetal development (growth and differentiation) with survival as a newborn infant is the only marker of fetal biology that assesses the effect of toxic agents on the fetus as a whole. Nonetheless, biomarkers have obvious potential for the developing fetus, although a number of problems make their use fraught with difficulty (Table 3). For instance, the fetus is not quite as accessible as one might sometimes wish, and obtaining fetal cells, tissue, or amniotic fluid for marker

Table 3. Unique problems of the use of fetal biomarkers.

Accessibility
Hormonal differences
Metabolic differences
Species differences
Gestational age
Litter size
Mass in relation to mother
Maturity at birth

Table 4. Diagnostic ultrasound and biological markers.

Fetus	Placenta
Maturational or morphometric biomarkers	
Gross malformations	Size
Estimated fetal weight	Maturity
Head: biparietal diameter, circumference	
Abdomen: circumference	
Head/abdomen: circumference	
Femur: length	
Heart: R/L ventricle, malformations	
Functional or physiological biomarkers	
Brain: cerebral blood flow	Blood flow: uterine
Cardiovascular: heart rate, cardiac output, umbilical blood flow, umbilical systolic/diastolic	Blood flow: umbilical
Respiratory: breathing movements, somatic movements	

determination can be fraught with difficulty. Despite this relative inaccessibility, one can measure a number of fetal physiologic variables, but most of these are rather nonspecific, imprecise, and insensitive as markers of toxicity. Also, there are major hormonal differences between the fetus and adult (some of which can be used to advantage) and differences in metabolism. A further complication is that fetal responses can vary widely among species, so that a given marker in one species may not have application to other species. Additionally, the fetus consists of a multitude of cell types, each with unique responses to various agonists, and the reactions of the several cells and tissues can vary with their maturity and the stage of development. As a corollary, the fetal maturity for the various species can vary widely at birth. In addition, while much is known of the effects of various chemicals and toxins, little is known of the critical levels, critical periods during development, or how biological variation affects the various responses. A final problem is that although one may observe and measure rather gross effects, there are few indices of more subtle cellular and subcellular changes.

In light of these considerations, this review will focus on individual components of the fetal system, i.e., organ, tissue, and cell, and consider how biological markers might serve at several hierarchical levels as indices of exposure or toxic effect.

Validated Techniques for Biological Markers

Ultrasonography

Of the tools currently being used successfully for fetal diagnosis, few have attracted the attention of diagnostic ultrasound. During the past decade, the limits of resolution of antenatal ultrasound have increased dramatically from the detection of gross defects such as anencephaly to subtle abnormalities of the fetal brain, heart and vascular system, kidneys, and other organs (Table 4) (10). The accuracy of high-resolution ultrasound is of course the critical issue. Anencephaly can be diagnosed with 100% certainty (11). Anomalies (such as alobar holoprosencephaly with absent midline cerebral structures and related hypotelorism) manifested by two or more embryologically related markers, each of which is detectable by ultrasound, can be diagnosed reliably (12). This is in contrast to the accuracy in instances of single abnormalities of dimension (such as microcephaly) or those in which the structure of interest is visualized

inconsistently. Unfortunately, although instrument resolution and operator skill have improved, few studies have rigorously examined the diagnostic accuracy for various anomalies.

An area in which ultrasound has advanced rapidly is in the diagnosis of fetal size, and it is hoped, maturity. For instance, certain morphometric measurements have been found to be useful, including biparietal diameter and circumference of the head, abdominal circumference, and the length of the femur and other long bones.

Ultrasound is of value in the diagnosis of fetal heart defects and cardiovascular function (13). Combining real-time with static techniques (so-called M mode) has allowed detection of cardiac morphology, rhythm, chamber size, and pericardial effusion (13). In addition, pulsed Doppler technology allows the measure of velocity through cardiac valves and in major vessels. Doppler technology, combined with measure of diameter, permits a calculation of cardiac output and/or blood flow rate. More than 20 major malfunctions and malformations, including those of the genitourinary tract and other systems, are diagnosable with these techniques (14).

In addition to its diagnostic potential per se, ultrasound enhances the safety and effectiveness of other diagnostic methods such as amniocentesis, chorionic villus sampling, fetoscopy, and fetal blood sampling (15–17).

Amniocentesis

The ability to enter the amniotic cavity without appreciable risk to either mother or fetus has remarkably influenced obstetric care. Aspiration of a sample of amniotic fluid allows a number of diagnostic tests to be performed that are indicative of fetal well-being. Initially, amniocentesis was employed to estimate the concentration of bilirubin (and related pigments) in amniotic fluid, and thereby identify hemolytic disease. Currently, amniocentesis is primarily used later in gestation to determine the relative concentration of surfactant-active phospholipids released from the fetal lung

in an effort to assess the risk of a premature infant developing respiratory distress syndrome. In addition to allowing quantification of bilirubin concentration, the lecithin-to-sphingomyelin ratio, and individual phospholipids such as phosphotidyl glycerol, amniocentesis is becoming increasingly useful to identify a number of hereditary disorders. Measurement of abnormal biochemical processes has proven useful in the antenatal diagnosis of about 100 inborn errors of metabolism. Essentially all chromosomal anomalies, i.e., trisomy 21,13, and 18, and triploidy, are diagnosable from amniotic fluid samples (18,19). Also, α-fetoprotein levels can be measured in amniotic fluid and can suggest the presence of neural tube defects, congenital nephrosis, and trisomy 21 (20). In addition, analysis of cells from amniotic fluid may be used for the antenatal diagnosis of chromosomal abnormalities, genetic enzymatic defects, and DNA adducts.

Chorionic Villus Sampling

As with amniocentesis, this relatively new technique allows one to obtain cells that reflect the genetic constitution of the conceptus. Used during weeks 8 to 12 of the first trimester, diagnostic results from chorionic villus sampling are available within a few days to 2 weeks. Most commonly, a small sample of chorionic villi is aspirated through a flexible catheter which is passed through the cervix under sonographic guidance (21). Alternatively, an aspiration needle can be passed transabdominally (22). As with cells obtained via amniocentesis, the diagnosis of hereditable disorders may be made by enzyme assays, chromosome number or abnormality, and DNA analysis (Table 5) (23).

Several problems make chorionic biopsy more complicated than amniocentesis. The risk of interruption of pregnancy is not insignificant. In addition, contamination of the villi with maternal decidua is possible. Finally, in chorionic villi, chromosomal mosaicism appears to be more common than in amniotic fluid cells (19).

Table 5. Chorionic villus sampling.[a]

Enzyme analysis	Cytogenetic analysis
Tay-Sachs disease	Normal male (46XY)
Adreno-leukodystrophy	Normal female (46XX)
Farber's disease	Trisomy
GM_I gangliosidosis	Translocation
Hunter's syndrome	Mosaic
Hurler's syndrome	Triploidy
Infantile Gaucher's disease	Inversion
Lesch-Nyhan syndrome	Sex chromosome abnormality
Menke's syndrome	
Metachromatic leukodystrophy	
Methylmalonic aciduria	
Niemann-Pick disease	
Sanfilippo's syndrome	
Wolman's syndrome	
Zellweger's syndrome	

[a] From Jackson (23).

Fetoscopy

Considerable interest exists in instrumentation that allows direct visualization of the fetus and placenta without the risk of disrupting the pregnancy (24). The possibility of direct visualization of externally located fetal anomalies and the ability to sample directly tissue from the fetus or fetal blood vessels in the umbilical cord or placenta without appreciable risk to the fetus or mother holds great promise for the detection of biological markers, whether specific compounds per se, DNA adducts, or by the use of DNA probes. Currently, however, fetoscopy is a research procedure carried out in only a few academic institutions.

Fetal Blood and Tissue Sampling

Fetal blood obtained in utero can be used for the antenatal diagnosis of hemoglobinopathies, coagulation defects, metabolic and cytogenetic disorders, immunodeficiencies, and infections (25,26). Originally, such specimens were collected by direct visualization of umbilical or placental vessels through a fetoscope. More recently, percutaneous sampling of fetal cord blood with use of a needle under sonographic guidance has allowed fetal blood sampling with less risk to the unborn infant. The availability of this blood permits the diagnosis of many conditions and the detection of various biological markers (16).

Fetal skin and liver have been sampled successfully and used for histologic and biochemical studies when amniocentesis or fetal blood sampling could not provide the necessary information. Biopsy instruments have been introduced under both fetoscopic and ultrasound guidance to collect these samples. Although classically genetic disorders (such as epidermolysis and ichthyosis of the skin and glycogen storage disease and ornithine transcarbamoylase deficiency of the liver) have been diagnosed by this method, the tissue may be used for study with DNA probes, etc.

Examples of Validated Markers

There are a number of biomarkers, the use of which has been validated in conjunction with the techniques previously outlined. Some of these markers are of exposure, some of clinical effect, and some help to elucidate molecular mechanisms. Classic case studies have been presented on prenatal exposure to methylmercury (27,28), lead (29), fetal alcohol (30) and fetal tobacco (31) syndromes, and others.

Promising Markers

Markers for Chemical and Environmental Teratogens. Prior to about 1960, the vast majority of birth defects were regarded as genetic in origin. The fetus was believed to occupy a privileged site within the uterus, protected from the effects of environmental

agents to which the mother might be exposed. Although association between maternal rubella infection and abnormal fetal development was recognized in the early 1940s, not until 20 years later did reports suggest that prenatal exposure to the drug thalidomide was the cause of serious developmental defects. Since then a host of chemicals, drugs, and environmental agents have been implicated as teratogens. Thus, the pendulum has swung the opposite direction, so that virtually every drug and chemical is suspected of being the cause of a congenital malformation. Table 6 lists some chemical teratogens, classified by their relationshiop to fetal abnormalities (3,32,33). Some drugs have potential adverse effects on the fetus, other drugs have a question-

able relationship to fetal anomalies, while for others, including several classed as "litogens" (34), no relationship to fetal abnormalities has been demonstrated (Table 6). Table 7 lists some drugs with potentially adverse effects on the newborn infant.

Because of a number of circumstances, the vast majority of pregnant women are exposed to a variety of agents. The scope of the problem is mind boggling, as illustrated by the NIH Collaborative Perinatal Study finding that the average pregnant woman takes five drugs (including nutritional supplements) during the course of her pregnancy, and about one-half of the total drug consumption occurs during the period of organogenesis, i.e., during the first trimester (3,35). Although only 2 to 3% of developmental defects are known to be due to drugs and chemicals and 65% are of unknown origin, one cannot be sanguine with these figures, for the overwhelming size of this "unknown" category only reflects our ignorance and stresses the need for markers that will help to identify specific chemicals and their relation to given defects.

Examples of environmental chemicals that can harm the fetus and can be particularly troublesome because of their ubiquity and unsuspected presence are chlorbiphenyls, laboratory solvents, hapthalene, and organic mercury. Again, the need for biomarkers of exposure/ effects for these and other chemicals is enormous.

Heat-Shock Proteins. Heat-shock proteins are produced by many cells in response to hyperthermic, hypoxic, or chemical stress. These proteins are of several size classes (usually 20–100 kilodaltons), and are synthesized by normal cells. Although the functional significance of these polypeptides is unknown, some evidence suggests that they aid in the cells' tolerance to

Table 6. Relationship of chemical teratogens to fetal abnormalities.[a]

Effect	Compound
Definite relationship	Alcohol
	Diphenylhydantoin
	Folic acid antagonists
	Inorganic iodides
	Lithium
	Organic mercury
	Retinoids—Vitamin A, Acutane
	Sex steroids (including diethylstilbestrol)
	Streptomycin
	Tetracyclines
	Thalidomide
	Thiourea compounds
	Trimethadione
	Warfarin
Probable relationship	Alkylating agents
	Chlorobiphenyls
	Diazepam
	Kanamycin
Questionable relationship	Amphetamines
	Chlordiazepoxide
	Clomiphene
	Diphenhydramine
	Ethionamide
	General anesthesia (chronic exposure)
	Gonadotropins
	Haloperidol
	Lysergic acid diethylamide (LSD)
	Meprobamate
	Metronidazole
	Oral hypoglycemic agents
	Penicillamine
	Phenothiazines
	Quinine
	Cigarette smoking
No relationship	Bendectin
	Corticosteroids
	General anesthesia (short-term exposure)
	Heparin
	Isoniazid
	Meclizine
	Penicillin
	Spermatocides
	Sulfonamides

[a] From Jones and Chernoff (32); Shepard (35); and Simpson et al. (33).

Table 7. Drugs with potential adverse effects on the neonate.

Drug	Potential adverse effect
Azathioprine	Decreased immunologic competence
Cannabis	Neurobehavioral abnormalities
Chloramphenicol	Gray syndrome
Diazepam	Floppy Baby syndrome
Heroin	Pre- and postnatal growth retardation, microcephaly
Hexamethonium	Paralytic ileus
Lithium	Cardiac malformations
Naphthalene	Hemolysis (G6PD deficiency)
Narcotics addiction (cocaine, etc.)	Withdrawal
Nitrofurantoin	Hemolysis (G6PD deficiency)
Oxytocin	Hyperbilirubinemia
Phenobarbital (excess)	Neonatal bleeding
Propranolol	Hypoglycemia and bradycardia
Quinine	Thrombocytopenia
Reserpine	Nasal congestion
Salicylates	Platelet dysfunction
Cigarette smoking	Intrauterine growth retardation
Sulfonamides	Hyperbilirubinemia
Thiazides	Thrombocytopenia and electrolyte imbalance
Warfarin	Bleeding disorders

excessive temperature and other stress. Several teratogens, including diphenylhydantoin, diethylstilbestrol, griseofulvin, and coumarin, have been demonstrated to result in the synthesis of certain proteins of the heat shock class by amniotic fluid cells (36). Potentially, specific polypeptides for these or other toxins may be produced by cells in amniotic fluid or fetal blood, which could be quantified by appropriate radioimmunoassays.

Promising Techniques for Biological Markers

Fetal Body and Breathing Movements

Normally, during the second half of pregnancy, expectant mothers are cognizant of frequent movements by the fetus. In normal pregnancies such movements increase in number from about 30 per 12 hr at 24 weeks of gestation to about 130 at 32 weeks, and then to about 100 at term; however, there is considerable normal variation in these values (37). The fetus that moves consistently is usually healthy. In contrast, a sudden decrease in this activity is an ominous sign suggesting fetal compromise. In those instances of cessation of fetal movements, the fetus often dies within the next 24 hr (38). Decreases in fetal movements have been associated with hypoxia and other conditions.

Fetal breathing activity can be detected by ultrasonography and other techniques by 11 weeks. By 16 weeks these breathing movements are sufficiently intense to move small amounts of amniotic fluid in and out of the respiratory tract. During the third trimester, such movements average about 50/min and are episodic. In sheep, much of the activity occurs in association with periods of rapid eye movements and high frequency, low voltage, electrocortical activity (39). The frequency of fetal breathing movements decreases in association with hypoxemia, asphyxia, hypoglycemia, maternal smoking, ethanol ingestion, and other stresses. Thus, these breathing and body movements are an index of fetal well-being. Changes in these functions, however, are rather nonspecific and thus are rather crude biological markers.

Electronic Fetal Heart Rate Monitoring

Antepartum and intrapartum surveillance of fetal well-being with early detection of fetal distress is most commonly accomplished by monitoring the fetal heart rate. During uterine contractions, uteroplacental blood flow temporarily decreases. In instances of fetal compromise by intrinsic fetal disease, placental insufficiency, umbilical cord compression, maternal disease, maternal hypoxemia or hypotension, or the administration of certain drugs, the heart rate will decrease, usually during or after a contraction. In addition, there may be a decrease or loss of the normal beat-to-beat heart rate variability.

The field of fetal heart rate monitoring has developed an extensive literature with its own terminology. Unfortunately, the wealth of empirical observations and associations has not been matched by a deep understanding of the physiologic basis for the phenomena observed. Although of proven usefulness in the detection of fetal distress, changes in heart rate and beat-to-beat variability are sufficiently nonspecific as to make their role as biomarkers of limited value.

Biophysical Profile

Evaluation of several parameters of fetal well-being have been combined into the biophysical profile. By the use of electronic monitoring, fetal heart rate reactivity is determined, and with diagnostic ultrasound, fetal gross body movements, muscle tone, breathing activity, and amniotic fluid volume are evaluated with an Apgarlike score (40). Again, although apparently of value empirically, each index is nonspecific and of diagnostic value in only a limited sense.

Nuclear Magnetic Resonance

Organ and tissue imaging using nuclear magnetic resonance (NMR) is becoming available in tertiary and many other medical centers. Several reports of this technique during pregnancy suggest its usefulness (41–43); however, the safety of the procedure during gestation has yet to be established. The high magnetic fields required could potentially affect embryonic and fetal development, particularly in pregnant individuals who work with the instrument on a long-term basis. In experimental animals the use of ^{31}P-NMR to determine functional metabolic correlates, temporal relationships, and intracellular actions of chemicals in organs such as the heart, liver, and placenta has great potential. The use of paramagnetic ions for specific visualization of the placenta and conceptus may have limited application (44).

Molecular Probes

Perhaps one of the most exciting emerging new areas of antenatal diagnosis is the use of DNA probes that reveal genetic markers near specific genes (19). For instance, just during the past year, DNA probes have been applied to the prenatal diagnosis of cystic fibrosis, and predictive testing began for individuals who might carry the gene for Huntington's disease (Table 8). In addition, a portion of the gene for Duchenne muscular dystrophy was isolated, and the recessive oncogene responsible for familial predisposition to retinoblastoma was discovered. Such a genetic marker is a segment of DNA that lies near a disease gene that has not been identified. In instances where the marker is consistently inherited by victims of the disease, it signals that the defective gene must be near the marker. Potentially, with DNA probes and genetic markers it would be possible to detect most of the more than 3000 conditions caused by single gene mutations. Recent advances in the amplification of desired DNA sequences by as much

Table 8. Disorders diagnosable by analysis of cellular DNA.

Validated	Probable
Sickle cell anemia	Fragile X syndrome
β-Thalessemia	Adult onset polycystic kidney disease
α-Thalessemia	
Factor VIII deficiency	
Factor IX deficiency	
Phenylketonuria	
α-Antitrypsin deficiency	
Huntington's disease	
Antithrombin III deficiency	
Ornithine transcarbamoylase deficiency	
Duchenne's muscular dystrophy	
Argininosuccinic acid dehydrogenase deficiency	
Osteogenesis imperfecta type II	
Congenital adrenal hyperplasia	

as a millionfold make such sequences much easier to detect.

The applications of molecular biology to clinical medicine will drastically change the approach to the diagnosis of disease (19). Unique sequence DNA probes and probes for restriction-fragment-length polymorphisms are being generated at an ever increasing rate. Within a few years, all monogenetic human diseases will have molecular determinants, so that clinical diagnosis will radically change from an emphasis on phenotype to that of molecular genotype. Molecular diagnosis, even during prenatal life, is possible by the use of three different but related techniques, e.g., restriction enzymes, oligonucleotide probes, and restriction-fragment-length polymorphisms (RFLPs). Briefly, highly specific restriction endonucleases cut DNA at certain base sequences. When altered by mutation, the DNA is severed into fragments of a size different from normal. For instance, in sickle cell anemia the mutation results in loss of a restriction enzyme recognition site, so that the resulting single DNA fragment is the size of the two fragments from a normal beta-globin gene. The homozygous (SS) and heterozygous (SA) states of sickle cell anemia can be differentiated by this method. Abnormalities of the genes for growth hormone and 21-hydroxylase (the deficiency of which results in congenital adrenal hyperplasia) are examples of other conditions diagnosable by this approach.

In instances where the precise DNA mutation is known but the mutation is not discriminate for a restriction enzyme cut, oligonucleotide probes representing the normal and abnormal sequences may be used to identify normals, heterozygotes, and homozygotes for the mutation in question. As increasing numbers of normal and mutated gene sequences are identified, the practicality of this technique will increase. Restriction-fragment-length polymorphisms, the third tool to be developed, has been used to diagnose a number of conditions, including Huntington's disease, phenylketonuria, factor VIII and IX deficiencies, and some cases of beta thalassemia.

The versatility of the DNA technology is enormous, since a condition such as congenital adrenal hyperplasia potentially may be diagnosed by any one of these three methods. As increasing numbers of these polymorphisms are recognized and restriction enzymes are developed, every monogenetic disorder is potentially diagnosable by this technique. As the molecular map of the human genome is completed, functional correlations will evolve that may provide insights into the basic pathogenesis of most disorders, including those caused by chemical mutagens and physical factors, e.g., irradiation. The impact of molecular biology on clinical medicine and diagnosis during the next decade is beyond our imagination. Presumably, all diseases and toxic conditions will be defined in molecular terms and diagnosable from a few microliters of blood. This scenario may be upon us before we have the time to grasp the implications of this new technology (17).

From the standpoint of xenobiotics, DNA probes may be used to detect chemical or food contaminants. Such DNA-probe-based tests could replace current assays on the basis that they are much more sensitive and require less time to perform. R. Everson has reviewed the potential use of DNA adducts in prenatal diagnosis elsewhere in this volume (45).

A caveat should be noted regarding tests that employ such DNA probes or DNA adducts. A potential pitfall is that as these tests become more widely used, particularly if commercial kits become available, quality control may suffer. The assurance of accuracy is essential. Reliability may suffer because of problems such as incomplete DNA digestions, faulty hybridizations, contamination, and mislabeling. Even in the best of hands, the interpretation of these tests and the associated counseling of families at risk require great experience and commitment.

Finally, there exists the problem of validation of these tests when no confirmatory assays exist. These and related issues accentuate the problem that although these revolutionary developments in DNA probes have enormous potential as biomarkers in antenatal diagnosis, many challenges lie ahead.

Research Gaps/Needs

When this committee was impaneled, we were given several objectives: to identify and evaluate biological processes that may yield useful markers, to develop guidelines for the validation of biological markers, to suggest strategies for the use of markers in human health and environmental protection, and to identify promising areas for future research on such markers. Certainly, in terms of the fetus, each of these goals is a major research need.

The use of biological markers to assess fetal risk presents a paradox. During the past decade a number of techniques have become available both by invention and improvement of existing methods. At the same time increasing numbers of chemicals have been recognized to have teratogenic and mutagenic potential. Nonethe-

less, essentially no specific biomarkers are currently available to indicate that exposure to a given xenobiotic is directly associated with a cellular, subcellular, or pharmacodynamic event.

I would suggest, therefore, five major areas for research. First, as has been voiced by many contributors to this symposium, is the need for specific biological markers. Whether chemical detection in fetal blood or tissue, the extraembryonic membranes, amniotic fluid, or maternal blood, or by the use of molecular techniques, such biomarkers are essential. Second, validation of the accuracy, reliability, and predictability of these markers will be required. Third is the need to develop new approaches and techniques, including more ready accessibility to the fetus. Fourth is the necessity of developing strategies for the use of biomarkers for protection of the fetus, and fifth is the requirement for increased understanding of the fundamental mechanisms whereby normal embryonic and fetal development proceeds and how chemicals and other xenobiotics act on cellular and organ systems.

The achievement of these research goals will not be easy, nor will they come cheaply. As with other great efforts—the mapping of the human genome, the understanding of the molecular basis of disease, and the regulation of growth and differentiation during normal development—these objectives will require a major research endeavor. In addition, the developments that will allow the achievement of these goals cannot be predicted or planned by a committee. As with most scientific advances, first-class discoveries only can originate from the minds of scientists unfettered by dogma or preconceptions who are allowed free range for their ideas.

In conclusion, the physiological assessment of fetal compromise by markers of toxic exposure is a goal worthy of the biomedical research community. Priorities need to be established regarding such research in humans and laboratory animals. Studies in pharmacokinetics and pharmacodynamics need to be conducted in various organ systems, tissues, and cells.

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